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THE MYXOMATOGENIC VIRUS. CONTRIBUTION TO THE STUDY OF CAUSATIVE
FACTORS OF DISEASE THAT ARE OUTSIDE OF THE RANGE OF VISIBILITY. ¹).

Centralblatt fuer Bakteriologie, Parasitenkunde, u. Infektionskrank-
heiten. Abt. I: Medizinisch-Hygienische Bakteriologie und Tierische
Parasitenkunde (Central Bulletin of Bacteriology, Parasitology, and
Infectious Diseases. Series.I: Medico-Hygienic Bacteriology and Ani-
mal Parasitology), Jena (Germany), Vol. 23, No. 20, Jun 1898, pp
865-873.

Prof. G. Sanarelli, Director, Insti-
tute of Hygiene, Montevideo

1. Are There Bacteria in Nature Which Cannot be Detected Under a
Microscope?

At present there is general agreement to the effect that viru-
lence is a biological function of lower organisms. However, the
causal relationship between microorganisms and a considerable number
of infectious diseases has not yet been established. Furthermore, the
diseases in question differ in regard to their nature, course, and un-
usual characteristics from those of the usual infectious type associated
with bacteria, which we recognize as such even when a specific germ
that is immediately apparent has not yet been discovered.

Rabies and syphilis belong to this class of virulent diseases.

The properties of their viruses, their syndrome and course,

¹) Report presented at the 9 th International Congress of Hygiene and
Demography, Madrid, 10-17 Apr 1898

the inner lesions associated with them, the outward symptoms, the mechanism of their anatomical propagation and of their incubation and hereditary transmission definitely indicate the presence of a pathogenic factor for these two diseases, the biology of which, however, cannot be deduced unless we reject the bulk of our knowledge pertaining to general criteria concerning the nature of organized viruses discovered hitherto .

➤ Because it is improbable that nonorganized causative factors of infection exist in nature, one is forced to assume that some diseases are caused by organisms which are so small that they are scarcely visible to the human eye, although aided (such as, for instance, the bacterium of peripneumonia discovered some time ago by Nocard and Roux), or else remain completely invisible.

The new disease with which this report deals furnishes an opportunity to consider this matter further () ←

2. Myxomatosis of Rabbits.

This disease broke out without apparent reason among rabbits of the Institute of Hygiene in Montevideo in the beginning of 1896. Its symptoms were as follows:

Rabbits that were perfectly healthy up to that time exhibited suddenly symptoms of a catarrhal blepharoconjunctivitis on both eyes.

After 24-48 hrs this initial lesion became so severe, that the strongly inflamed and swollen eyelids were closed completely. The eyeball was pressed back into the socket and the slit between the eyelids was filled with a thick, profuse, catarrhally suppurative secretion.

At the same time small subcutaneous tumors varying in size developed in various parts of the body, affecting particularly the ears and extremities.

Almost simultaneously the head of the animal began to change its shape: the mouth and nose became so thick that they assumed a lionlike appearance. The openings of the rectum and of the genital and urinary organs became acutely inflamed. The breast nipples of females became hypertrophic.

In general, the diseased rabbits exhibited in addition to neoplastic tumors dispersed over the whole body surface a hyperplastic process in all organs at locations in which the skin tissue changed into mucosa .

When this stage was reached, the rabbits lived only for a few days (2-5) longer, while the symptoms described increased in gravity. At the time of death, the animals looked deformed and repellent.

The results of postmortem dissections were generally as follows: subcutaneous tumors of gelatinous appearance with elastic consistency and a profusion of blood vessels; hypertrophy of lymph glands, orchitis, and swelling of the spleen.

3. Histological Lesions in Myxomatosis.

Fixation of anatomical specimens, which altered readily, had to be carried out by using Mueller's or Flemming's liquid or else mercuric chloride.

A microscopic examination of neoplastic tissue and of organs which showed anatomical changes disclosed the following facts.

The subcutaneous tumors consisted of typical myxomatous tissue with a predominance of star-shaped elements and with numerous capillary blood vessels.

The swelling of the eyelids consisted of a huge accumulation of newly formed myxomatous elements, which increased the thickness of the eyelids by a factor close to ten.

The swelling of external orifices, which was one of the most specific aspects of the disease, was due predominantly to the

presence of tissue of edematous appearance which consisted of the myxomatous elements described above.

The hypertrophy of the spleen and of the lymph glands was also caused by the presence of newly formed myxomatous tissue as well as hemorrhagic infiltration.

In general, the specific nature of the histological lesions was the same in all organs affected.

Myxomatous tumors associated in a regular manner with definite organs were present.

4. The Myxomatogenic Virus.

What is the nature of the specific agent responsible for this strange pathogenic process?

Every search for a bacterial or other parasitic form of life with the application of all techniques that can be applied at a laboratory at present remained unsuccessful. Although the explanation can be advanced that the causative factor could not be detected only because our methods of investigation were inadequate, I still conclude that the etiological factor does not belong to any form of organized life which we are at present accustomed to regard as the cause of specific diseases.

Nevertheless, infectious myxomatosis is transmissible without restriction from one rabbit to another; a drop of blood, a particle of a tumor, a trace of secretion from the eyelids, or a particle of intestine proved equally virulent in this respect.

On the other hand, urine (either clean or containing hemoglobin), pleural secretion, and the humor aqueus did not transmit the disease.

Experimental transmission of the disease could be carried out subcutaneously, endovenously, by way of the stomach, or endocularly

a) Subcutaneous Infection.

Infection took place in every instance when a small amount of

blood or a particle of tissue or of an organ derived from a dead animal was introduced under the skin.

After an incubation period of 4-5 days, during which the animal was perfectly healthy and gained weight, a myxomatous tumor formed at the site of the inoculation, which increased in size until the death of the animal. At the same time bilateral blepharoconjunctivitis appeared, which developed in the manner described above, and hyperplastic phenomena developed at body orifices.

The older the animal, the slower and more noticeable was the development of external symptoms.

Death usually occurred on the 10 th day after inoculation of the virus.

b) Endovenous Inoculation.

Endovenous inoculation could be carried out by injecting a trace of infected blood or a small amount of edematous liquid from a specific tumor into a vein at the edge of an ear.

The results were the same as those described above. After the usual incubation period, inflammation of the eyelids set in and all other symptoms followed.

c) Infection by Way of the Stomach.

It could be produced in a rabbit by making it swallow a piece of some /infected/ organ, e. g., the spleen, the liver, or the kidney.

The onset and course of the disease as well as the results of the dissection were the same as after subcutaneous or intravenous inoculation.

Contrary to what one might have expected, the digestive tract appeared completely normal in the autopsy, just as in all other cases.

d) Endocular Infection.

A trace of virulent liquid injected into the front chamber of the eye sufficed to transmit the infection with certainty. After an incubation period of 4 days iridocyclitis of the eye into which injection had been carried out developed and shortly thereafter all characteristic symptoms of the disease appeared.

5. Ways in Which the Virus of Myxomatosis Spreads.

My studies showed that the disease was transmitted to spontaneously infected rabbits by the catarrhal-suppurative secretion from the eyes and nose.

To reproduce this natural way of contagion experimentally, it was sufficient to touch the conjunctiva of a healthy rabbit with a platinum loop that had been drawn over the conjunctiva of a sick animal. On the 5 th day the infected eye suddenly developed the specific blepharoconjunctivitis, while on the 8 th day the infection spread to the other eye. On the 9 th day at the latest, the infected animal died with all known symptoms of the disease.

It is unnecessary to repeat that one cannot detect in the infectious eyelid secretion or isolate from it any pathogenic germ or parasite.

An attempt was made to establish the time required for the virus that had penetrated into the body to spread into the blood and into other organs. It was found that in rabbits infected by the way of the stomach the blood became virulent after 48 hrs and in intravenously infected rabbits after 24 hrs.

Thus, several days before specific symptoms of the disease developed and while the animal apparently still enjoyed perfect health in the incubation period, the organs and the blood were already saturated with the virus, which was present in the highest concentration, just as is the case of the whole secondary or latent period of infection with syphilis.

Upon spontaneous coagulation or centrifuging, the blood serum was separated from the fibrin and morphological elements of the blood. Injections to rabbits indicated that both the coagulated fibrin and the optically pure, completely sterile serum had the same capacity to infect.

6. Some Biological Properties of the Myxomatogenic Virus.

It was difficult to determine the concentration of the virus in the blood of sick animals; the results obtained varied considerably.

When 1-2 drops of blood were introduced into a 200 cc dish with meat broth, the latter acquired the capacity to infect in a small dose (1 cc) even when the blood collected at the bottom of the dish and did not visibly mix with main amount of liquid, which remained clear and colorless.

It is understandable that this was not due to propagation of the virus, if only because transfer /of the capacity to infect/ from one dish with meat broth into another did not succeed.

But the myxomatogenic virus could be reinforced by passage through several rabbits.

The disease has been passed without interruption from rabbit to rabbit in my laboratory from the beginning of 1896, when it was originally discovered, until the day of writing (Mar 1898).

As a result of repeated passages, the virus became more virulent, so that the duration of the disease decreased and its symptoms were considerably simplified.

At present the myxomatogenic virus kills rabbits already in 5 days, without giving them time to develop important external symptoms.

In cases of this type the only external symptoms of consequence are strong reddening of the conjunctiva and of the free edges of the eyelids, which develop exactly on the 4 th day, i. e., 24 hrs before

death.

The virulence of the virus was reduced as a result of aging or of the addition of antiseptics.

The myxomatosis virus contained in the blood was preserved for a long time by keeping the blood in Pasteur pipettes or in test tubes that contained a 1⁰/₁₀₀ solution of potassium oxalate. The blood remained liquid in this solution, so that it could be used easily for experiments.

However, the virulence decreased with increased time of storage of the blood.

It is impossible to derive a general rule, because the results of 34 experiments varied somewhat. However, the blood kept in Pasteur pipettes was still virulent after 40 days and that mixed with the potassium oxalate solution still killed after 50 days of storage.

The attenuation of the virus as a result of aging of the blood was manifested by a longer duration of the disease, in consequence of which all specific lesions characteristic for the disease developed more completely and were more severe.

The effect of antiseptics was studied by mixing rapidly 1 cc of their solutions with 1 cc of blood that was still liquid after being withdrawn from the heart of an animal immediately after death.

The effects of boric acid (3%), phenol (2%), mercuric chloride (1⁰/₁₀₀), pure Gram's liquid, formaldehyde (5%), and potassium permanganate (2⁰/₁₀₀) were investigated.

Contact of the virus for 6 hrs with the solutions mentioned had no other effect besides a slight reduction of virulence, which was expressed only in a longer duration of the disease induced by the virus.

Thus, the myxomatogenic virus exhibited a resistance to the most effective antiseptics known at present to a degree not shown

by any other germ constituting an organized form of life.

On the other hand, moist heat of 55° applied for a few minutes sufficed to destroy the virulence of all products capable of transmitting infection with the disease.

7. Myxomatosis in a Dog.

The myxomatogenic virus produced the disease syndrome regularly in rabbits only. Mice, Guinea pigs, monkeys, and poultry were generally resistant to the disease.

Experiments on dogs were successful in only one case, which is worth describing briefly because of the very interesting result obtained.

Rabbit blood in amounts of 5 cc was administered to a bitch of mixed breed weighing 11 kg, initially on 1 Oct 1896 and then again at irregular intervals of 15-20 days.

On 4 Nov 1896, swelling of all breast nipples developed suddenly. The nipples became as large as nuts in several days and acquired a cancerous appearance.

At the same time, numerous regions affected by alopecia appeared over the whole skin surface, particularly on the stomach, the chest, and the front legs.

The surface of the latter parts of the body soon became totally devoid of hair, while the skin appeared swollen and congested and was covered with a rash having the form of a diffuse, pustulous erythema.

The tightly stretched skin broke in an increasing number of places, while bleeding developed all over it.

On 8 Jan 1897, the tumors on the mammary glands were extirpated together with some pieces of skin. Scar development on the wounds proceeded slowly.

A histological study of the tumors showed that they consisted of

neoplastic tissue composed of major elements of epitheloid appearance, similar to a cancrroid or rather one of the types of proliferating dermatitis or epidermitis that are observed in leprosy, elephantiasis and certain inflammations of the skin and mucosa (Virchow's pachydermia).

The extensive swelling and excessive tendency of the skin to break were apparently due to neoplastic formation of myxomatous tissue under the epidermis. This tissue was rich in blood vessels and very similar to that observed in rabbits.

The bitch died on 3 Jun 1897 after exhibiting paresis and elephantiasis of the front legs as well as alopecia of the total skin surface accompanied by light hemorrhages. Anatomical and bacteriological investigations did not disclose anything.

8. Experiments on Human Beings.

A complex of symptoms so striking in etiological, symptomatic, and anatomic respects reminded me of certain human diseases (dermatose ophthalmias) for which no specific, organized causative factors had yet been discovered notwithstanding certain proofs for the infectious nature of the diseases.

In order to complete my study of the comparative pathological aspects of the disease, I decided to investigate the effect of the myxomatosis virus on the human organism.

The virus was used in the form of completely pure serum of rabbit blood that had been withdrawn aseptically from the animal a day before its death.

This serum, which in rabbits produced the disease with a lethal outcome even when used in a very small dose, was kept for 24-36 hrs in a warming chamber. It remained colorless, clear, and completely sterile, just as normal rabbit serum obtained on spontaneous coagulation of the blood.

The results of two experiments on human beings were as follows. The serum of rabbits with myxomatosis, on being injected in a dose of 5-6 cc into the connective tissue under the skin in the gluteal region, produced congestion phenomena in the conjunctiva accompanied by edematous swelling and pronounced pain sensation in the eyeball.

These phenomena disappeared rapidly after injections of the virulent serum had been discontinued.

9. Attempts at Vaccination and Serum Therapy .

Experiments on the vaccination of rabbits by applying various methods in use (attenuation of the virus with heat and antiseptics, administration of small doses, etc.) did not lead to any results. All attempts made hitherto at the vaccination of rabbits against the virus of myxomatosis remained unsuccessful.

During the continuous two-year long passage of the disease from rabbit to rabbit at the institute in Montevideo, two rabbits that had been injected with the virus and exhibited all external symptoms of the disease (ophthalmia, swelling of body orifices) recovered subsequently.

The fact that these rabbits had acquired immunity was confirmed by multiple and profuse injections of virulent blood, which was tolerated perfectly well.

The serum of these rabbits as well as that of a dog injected for 10 mos from time to time with large amounts of virulent blood were tried on rabbits with the purpose of prophylaxis and therapy.

The results of these experiments were entirely negative.

Consequently, infectious myxomatosis also differed with respect to the mechanism of vaccination and the production of substances having specific curative properties from diseases caused by bacteria, because this is the first time that the serum of an animal completely immunised against an infectious disease was absolutely inactive

in the prophylaxis and therapy of the disease in question.

Montevideo, Mar 1898.